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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

This grant made possible the establishment of a molecular biology and biotechnology facility at the Hopkins Marine Station. The facility includes special use equipment - an oligonucleotide synthesizer and automatic micromanipulator/microinjector and general equipment for molecular biology - an autoclave and upgraded centrifuge facility.

The equipment is being used by members of 8 laboratories and involves studies on (1) heterozygosity, polymorphism and speciation of marine organisms, (2) identification of planktonic organisms with molecular probes, (3) physiological adaptation in marine plants and animals, (4) cell biology and neurobiology and (5) natural product chemistry.

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Office of Naval Research Final Report  
Multi-User Facility  
Performance Report Grant #N00014-89-J-1280

Department of Defense  
University Research Instrumentation Program

Principal Investigator: David Epel

Grantee: Hopkins Marine Station  
Stanford University

Title: Multi-user Facilities for Molecular Marine Biology and Biotechnology

Start Date: 9-1-88

**Project Objective:** The aim of this facility is to provide equipment and technology to (1) ask previously unanswerable but *classical* questions in marine biology with the new approaches of molecular biology; (2) integrate the new molecular biology approaches into ongoing marine biology problems; and (3) to bring biotechnology into marine systems.

**Accomplishments:** The ONR funds and also NSF monies from another grant, combined with Stanford University matching funds, were used to equip a new 3,000 sq ft facility for molecular biology research in the Blin's Building at the Hopkins Marine Station. Equipment purchased with the ONR grant includes (1) an Applied Biosystems DNA synthesizer, (2) an Eppendorf Micromanipulator/microinjector, (3) a large-capacity autoclave (Consolidated) and (4) renovation of two Sorvall Centrifuge and purchase of miscellaneous rotors and small microcentrifuges.

The facility has been in operation since May 1989 and actively used by individuals from several laboratories. Examples of the uses and a brief description of products are given below.

### **DNA Synthesizer**

This equipment is for synthesis of specific nucleotide sequences. The major use is that if one has a partial amino acid sequence of a protein, one can then prepare all of the numerous candidate sequences of DNA which encode for that specific protein. Because of redundancy in the genetic code, one has to make numerous candidate sequences and then utilize these to isolate the gene for the specific protein. This

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equipment, in combination with PCR, has immensely simplified the isolation of genes and is a major factor in the startling advances in molecular biology .

There are numerous ongoing projects utilizing this equipment. Some center on questions of heterozygosity of marine populations (Dennis Powers lab). For example, one project assesses the relatedness (or non-relatedness) of symbiotic zooxanthellae in corals. The DNA synthesizer is used to prepare universal primers for 18s r RNA. A similar approach assesses relatedness/variability in the ribosomal gene of various marine sponges.

Several projects in Power's lab are centered on molecular basis of thermal adaptations. These use oligonucleotide sequences, in combination with PCR, to probe intraspecies variation in specific genetic loci. Other studies use the synthesizer, in conjunction with PCR, to quantify levels of specific mRNA in response to thermal stress.

Another project, also in Power's lab, allows identification and description of the population biology of the mussel, *Mytilus edulis*. A cryptic population exists which can only be identified by a specific segment of rRNA, and this segment differentiates populations north or south of San Francisco Bay.

The synthesizer is also being used in Irving Weissman's lab to probe relatedness of tunicate populations. The Alberte lab is also a very heavy user of this equipment, examining relatedness/intermixing of seagrass (*Zostera*) populations.

Gene isolation studies are being actively pursued in the Gilly and the Alberte labs. Gilly's lab is interested in the  $\text{Na}^+$ -channel in squid axon, and the synthesizer has been used for generating sequences with homology to the squid  $\text{Na}^+$ -channel. Alberte's lab is using similar techniques for isolating the mitroge-nase gene in bacteria and algae and assessing environmental effects as well as field abundance of the organisms. Similar approaches are being used for the nitrate reductase gene.

### **Eppendorf Micromanipulator/Microinjector**

This equipment provides the most highly automated and reproducible method for injection of genes, primarily for producing transgenic animals, (abalone, tunicates and fish). It is also being used for patch clamp studies.

For example, Power's lab has two projects producing transgenic organisms, using the automated microinjection capabilities. Both center on integration of growth hormone into the genomes of marine organisms (a vertebrate and an invertebrate). Gilly's lab is using the micromanipulator component to carry out precise mapping of channels in the olfactory epithelium of squid. Epel's lab utilizes microinjection of pH-indicating dyes to assess the regulation of pH<sub>i</sub> in embryonic cells.

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## Autoclave

The autoclave is used for sterilization of media used for growing bacteria and bacteriophage as vectors for cloning of genes. It is also being used for sterile glassware necessary for tissue culture work and the preparation of monoclonal antibodies. All the aforementioned labs have projects dependent on this equipment. In addition, the lab of Stuart Thompson carries out tissue culture work for which the autoclave is a prerequisite. Finally, Jonathan Roughgarden's lab is producing monoclonal antibodies to barnacle larvae, and an autoclave is essential for this work.

## Sorval Centrifuges and Rotors

This equipment is necessary for preparation of cell fractions and extraction of nucleic acids used in numerous operations in cell and molecular biology and biotechnology. The equipment is used by the Powers and Gilly labs for the aforementioned molecular biology work. In addition, the Carl Djerassi lab uses this equipment for their studies on sterol synthesis in marine sponges.

## Summary of Projects:

Heterozygosity/polymorphism/identification and evolution of organisms with specific DNA or monoclonal antibody probes:

Simona Sorger - tunicates (Weissman lab)  
Robert Rowan - Zooxanthellae (Powers lab)  
Lani West - sponges/barnacles (Powers lab)  
Jeff Mitton - *Mytilus* (Powers lab)  
Kristi Miller - barnacles (Roughgarden lab)

## Genetic and physiological adaptation:

Doug Crawford - *Fundulus* (Powers lab)  
Jason Smith - numerous marine plants (Alberte lab)  
Richard Zimmerman - marine plants (Alberte lab)

## Cell biology:

Mary Lucero - squid olfaction (Gilly lab)  
Bill Gilly, Clay Armstrong -  $\text{Na}^+$ -channel (Gilly lab)  
Tony Morielli - signal transduction (Thompson lab)  
David Epel, Brigitte Ciapa - signal transduction (Epel lab)

## Marine natural products:

Russel Kerr - Sterols in sponges (Djerassi lab)

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